

**Title: "Method for preparing an interactive compound of an anilide derivative with a porous support by supercritical fluid"**

- 5 The present invention concerns a method of interaction of nanoparticulate anilide derivative with a porous support, by the technology of supercritical fluids, in particular that of CO<sub>2</sub>.
- 10 In 40% of cases new pharmaceutical molecules, with high added value, such as anilide derivatives, are insoluble or of low solubility in water, which is detrimental to their bioavailability. Increasing the specific surface area of powders allows their dissolution rate to be
- 15 improved.  
The bioavailability of active principles can be considerably enhanced, then, if their dissolution rate is improved.
- 20 The generation of fine powders with high specific surface areas by the technology of supercritical fluids has been used for a decade and a half.  
Two types of processes are conventionally employed: the RESS (rapid expansion of supercritical solution)
- 25 process, and the SAS (solvent-antisolvent) process. By modifying the operating conditions it is possible to control the morphology and the size of the particles formed from active substance.  
The advantages of using supercritical CO<sub>2</sub> as solvent
- 30 are several:
- possibility of working at low temperature (> 31°C) for active substances sensitive to heat,
  - solvency readily modifiable by acting on the parameters of the process (pressure, temperature, flow
  - 35 rate, etc.),
  - ready separation of the solvent/solute mixture by simple decompression,
  - chemical inertness of the solvent: nontoxic, nonflammable, noncorrosive,

- low cost in comparison with the organic solvents conventionally employed.

Within the pharmaceutical, cosmetics, and nutraceutical fields there exist a number of patents and publications relating to the microencapsulation of an active substance in a coating agent. Nevertheless, the majority of the processes described relate not to the improvement of bioavailability but rather to the adsorption of an active substance on a support.

Bertuccio et al. (*Drugs encapsulation using a compressed gas antisolvent technique* - Proceedings of the 4<sup>th</sup> Italian Conference on Supercritical Fluids and their Applications 1997, 327-334 - Ed. E. Reverchon) describe a process in which the active substance is suspended in a solution of biopolymer which acts as the support. This suspension, placed in an autoclave, is subsequently placed in the presence of supercritical CO<sub>2</sub> in order to desolvate it (extraction of the solvent by supercritical fluid) and to bring about the complexation of the support by supersaturation on the active substance. This process is a batch process in which the active substance is not precipitated by the supercritical fluid, since it is in suspension. The structure of the particles of active substance is therefore unchanged, which does not contribute to improving its dissolution in an aqueous medium.

An identical process is described by Benoît et al. in their patent application WO 98/13136.

Another technique of deposition of a support consists in dissolving said support in the supercritical fluid and then causing this support to precipitate on the active substance. For this purpose the active substance and its support are placed beforehand in a stirred autoclave and the injection of supercritical CO<sub>2</sub> dissolves solely the support (this implies that the

support is soluble in the supercritical fluid and the active substance is not), which is precipitated by modifying the pressure and the temperature within the autoclave. In this case the initial structure of the active substance remains unchanged, and it is difficult to control the active substance/support ratio obtained in the precipitated complex. This batch process is detailed in patent application EP 706 821 of Benoît et al.

The microencapsulation process described by Shine and Gelb in their patent application WO 98/15348 consists in:

1. mixing an active substance with an encapsulating polymer,
2. liquefying the polymer by passing in a flow of supercritical fluid,
3. carrying out rapid depressurization so as to solidify the polymer around the active substance.

This process is applicable only with an active substance and a polymer which are insoluble in the supercritical fluid. Consequently the active substance retains its original structure, which does not contribute to improving its bioavailability.

In patent application FR 2 798 863 of Perrut and Majewski, the active substance (kava-kava, curcuma, mixture of black pepper and sweet pepper), extracted beforehand with supercritical fluid, is precipitated in an autoclave containing a porous support. The porous medium studied is maltodextrin. The process is therefore one of simple inclusion in a porous support, without a step of diffusion in static mode of the active substance into its support. However, precipitation on a support is not sufficient to improve substantially the solubility of the active substance in aqueous medium.

The Tomasko group (Chou et al., *GAS crystallization of polymer-pharmaceutical composite particles*, Proceedings of the 4<sup>th</sup> International Symposium on Supercritical Fluids, 1997, 55-57 and Kim J.-H. et al., 5 *Microencapsulation of Naproxen using Rapid Expansion of Supercritical Solutions*, Biotechnol. Prog. 1996, 12, 650-661) mentions two processes of coprecipitation by RESS and by SAS with supercritical CO<sub>2</sub>. The active substance studied is naproxen, while the support is 10 poly-L-lactic acid (L-PLA). These two compounds are dissolved simultaneously in acetone before being precipitated by countercurrent injection of CO<sub>2</sub>, in the case of the SAS process. The complex thus formed is recovered after a wash phase. A mixture of naproxen and 15 L-PLA is placed in a chamber, from which the two compounds are extracted by the supercritical fluid and are precipitated in a second autoclave, as far as the RESS process is concerned. However, the precipitation or coprecipitation of an active substance and a support 20 is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Moreover, there again, no step of molecular diffusion in static mode in order to improve the interpenetration of the active substance with its support is described 25 in these two processes. Finally, the solubility of the active substance in an aqueous medium was not studied.

The same is true of the coprecipitation processes described by Sze Tu et al. (*Applications of dense gases* 30 *in pharmaceutical processing*, Proceedings of the 5<sup>th</sup> Meeting on Supercritical Fluids 1998, Tome 1, 263-269), Weber et al., (*Coprecipitation with compressed antisolvents for the manufacture of microcomposites*, Proceedings of the 5<sup>th</sup> Meeting on Supercritical Fluids 35 1998, Tome 1, 243-248) and Bleich and Müller (*Production of drug loaded by the use of supercritical gases with the Aerosol Solvent Extraction System (ASES) process*, J. Microencapsulation 1996, 13, 131-139).

Subramaniam et al. in their patent application WO 97/31691 developed an apparatus and a process starting from antisolvents which were close to the critical point and were supercritical, which allows particles to be precipitated and coated. The contact phase between the solution, the suspension containing the solute, and the supercritical antisolvent is performed such that it generates high-frequency waves, which divide the solution into a multiplicity of droplets. In this patent the particle size claimed is from 0.1 to 10  $\mu\text{m}$ . Additionally, coating processes are also described. The crystallizations of hydrocortisone, of poly(D,L-lactide-glycolide), of ibuprofen, and of camptothecin are described. However, the precipitation or coprecipitation of an active substance and a support is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Furthermore, this process does not describe a step of molecular diffusion in static mode, allowing the bioavailability of the active substance to be improved.

Tom et al. (*Applications of supercritical fluids in controlled release of drugs*, Supercritical Fluids Engineering Science ACS Symp. Ser. 514, American Chemical Society, Washington DC, 1992) report the first coprecipitation by RESS process of microparticles of lovastatin active substance (anticholesterolemic) complexed to a polymer, DL-PLA. The two compounds are placed in an autoclave, extracted with supercritical  $\text{CO}_2$ , and precipitated in a second chamber. The major drawback of such a process is the active substance/support ratio obtained in the complex. This is because this ratio cannot be selected precisely, since it is determined by the solubility of each of the two compounds in  $\text{CO}_2$  in the supercritical state. However, the coprecipitation of an active substance and of a support is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Furthermore, this process does not describe a

step of molecular diffusion in static mode, allowing the bioavailability of the active substance to be improved and, moreover, its solubility in an aqueous medium is not studied.

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A process for impregnating pharmaceutical actives is claimed in patent application WO 99/25322 of Carli et al. It breaks down as follows:

1. dissolution of the active principle by RESS
- 10 process,
2. contacting of the supercritical fluid containing the active principle with the crosslinked polymer,
3. impregnation of the crosslinked polymer in static or dynamic mode,
- 15 4. removal of the supercritical fluid.

Only active substances which are soluble in the supercritical fluid can be compared by this process, since the first step consists in extracting the active

20 principle with the supercritical fluid. Moreover, the process is not an inclusion process but a process of impregnation on a support, and no result is given concerning the improvement of the dissolution in an aqueous medium of the active principle thus prepared.

25 Finally, the impregnated polymer does not undergo a step of washing with supercritical fluid.

Fisher and Müller describe in their patent US 5 043 280 a process for preparing active substances on a support

30 with supercritical fluid. This process consists in contacting one or more actives with one or more supports in supercritical medium. For this purpose the actives and the supports are either precipitated or coprecipitated by SAS and/or RESS processes. The

35 compounds are obtained in sterile form. However, the precipitation or coprecipitation of an active substance and a support is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Furthermore, this process does not

describe a step of molecular diffusion in static mode, allowing the bioavailability of the active substance to be improved, and, moreover, its solubility in an aqueous medium is not studied.

5 Van Hees et al. (*Application of supercritical carbon dioxide for the preparation of a Piroxicam- $\beta$ -cyclodextrin inclusion compound*, Pharmaceutical Research, Vol. 16, No. 12, 1999) describe in their  
10 publication a process for including piroxicam in  $\beta$ -cyclodextrins using supercritical CO<sub>2</sub>. The process consists in placing a mixture of piroxicam and  $\beta$ -cyclodextrins (molar ratio 1/2.5) in a pressurized autoclave, which is left in static mode. Following  
15 depressurization, the mixture obtained is ground and homogenized before characterization.  
These analyses allow conclusions to be drawn concerning the degree of complexation of the piroxicam with the  $\beta$ -cyclodextrin, but do not provide any result  
20 concerning the improvement of the dissolution in aqueous medium of the piroxicam/ $\beta$ -cyclodextrin complex in relation to piroxicam alone. Moreover, the active substance used was not generated by supercritical fluid, and no step of washing the complex with  
25 supercritical fluid is performed.

Kamihira M. et al. (*Formation of inclusion complexes between cyclodextrins and aromatic compounds under pressurized carbon dioxide*, J. of Fermentation and  
30 Bioengineering, Vol. 69, No. 6, 350-353, 1990) describe a process for extracting volatile aromatic compounds and for trapping them by inclusion in cyclodextrins. Geraniol and mustard oil are extracted in this way by a RESS process and are vaporized in dynamic mode in a  
35 second autoclave containing a mixture of cyclodextrin and water. The influence of the parameters of temperature, pressure, and water content is studied by measuring the level of inclusion of the active substances in the cyclodextrins. The inclusion step

described in this publication is performed in dynamic and not static mode as claimed in the present invention. Moreover, this process does not include a step of washing of supercritical fluid. Finally, the  
5 solubility of the active substance in an aqueous medium is not studied.

Sze Tu L. et al. (*Application of dense gases in pharmaceutical processing*, Proceedings of 5<sup>th</sup> meeting on  
10 supercritical fluids, Nice, France, March 1998) describe in their publication how to perform precipitation by SAS of an active substance (para-hydrobenzoic acid) and polymers (PLGA - polylactide-co-glycolide - or PLA - poly-L-lactic acid). This  
15 coprecipitation is performed by two techniques; either with the polymer and the active substance in two different solutions; or else in the same solution. In both cases the two solutions, or the solution, containing the two components are treated by  
20 supercritical CO<sub>2</sub> SAS. However, the coprecipitation of an active substance and a porous support is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Moreover, this method does not describe a step of molecular diffusion  
25 in static mode, allowing the bioavailability of the active substance to be improved, and, moreover, its solubility in an aqueous medium is not studied.

The same is true of the coprecipitation processes  
30 described by Jung et al. in their patent FR 2 815 540. This is a process for fabricating very fine particles containing at least one active principle inserted into a host molecule, and also a device allowing this process to be implemented. This process consists in  
35 dissolving the active principle in a first liquid solvent, and a product formed from host molecules, of cyclodextrin or crown ether type, in a second liquid solvent. The solutions are subsequently contacted with a fluid at supercritical pressure, so as to cause the



molecules to precipitate, in an SAS process. The components, as in the process described by Sze Tu L. in the article cited before, can be dissolved in the same solvent. The results presented by Jung et al. do not claim any improvement in the dissolution rate. However, the coprecipitation of an active substance and a support of cyclodextrin type is not sufficient to improve substantially the solubility of the active substance in aqueous medium. Furthermore, this method does not describe a step of molecular diffusion in static mode, allowing the bioavailability of the active substance to be improved, and, moreover, its solubility in an aqueous medium is not studied.

Furthermore, none of the above cited prior art document describe a method of inclusion of an anilide derivative in a porous support.

The inventors of the present application have discovered, surprisingly, that a method comprising the steps of generating an anilide derivative by a supercritical fluid, mixing it with a porous support, followed by a step of molecular diffusion by the supercritical fluid in static mode and of washing with the supercritical fluid, makes it possible to prepare an interaction compound by very greatly increasing the solubility of the anilide derivative in an aqueous medium, and hence its bioavailability.

Indeed, the step of inclusion in static mode coupled with the phase of precipitation of the anilide derivative to its support has made it possible, surprisingly, to improve the dissolution of the anilide derivative in aqueous medium. Moreover, the third phase of washing in a supercritical medium, which consists in eliminating the residual solvents by passage of a flow of supercritical CO<sub>2</sub>, also makes it possible, surprisingly, besides the washing of the interaction

compound, to increase the dissolution following this step.

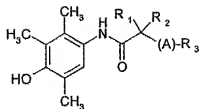
Moreover, these steps can be carried out batchwise or continuously, as is the case in particular for the diffusion and the washing. This makes it possible, therefore, to lighten the method relative to the conventional steps, which would be:

1. crystallization
- 10 2. solid/liquid separation
3. drying
4. inclusion in the support
5. micronization

15 The present invention accordingly provides a method for preparing a compound of interaction of an anilide derivative with a porous support, characterized in that it comprises the following steps:

- (a) mixing, advantageously intimately, the anilide  
20 derivative generated by supercritical fluid and the defined amount of porous support,
- (b) implementing a step of molecular diffusion by contacting in static mode a supercritical fluid with the mixture obtained in step (a) for the time required  
25 to improve the dissolution in an aqueous medium of the mixture obtained in step (a),
- (c) washing the interaction compound obtained in step (b) with a flow of supercritical fluid,
- (d) recovering the particles of the interaction  
30 compound thus formed.

By "anilide derivatives" is meant in the sense of the present invention any anilide derivative. Advantageously, it is a derivative of general formula I  
35 below:



I

in which:

- $R_1$  and  $R_2$ , which are identical or different, represent  
 5 independently of one another a hydrogen atom; a linear  
 or branched  $C_1$ - $C_6$  alkyl radical; an aromatic group such  
 as phenyl, naphthyl or pyridyl which is optionally  
 substituted by one or more  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy,  
 hydroxyl or halo groups,  
 10  $R_3$  represents a linear or branched  $C_6$ - $C_{15}$  alkyl chain or  
 a phenyl group which is optionally substituted by one  
 or more  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy, hydroxyl or halo  
 groups,  
 A represents a sulfur or oxygen atom or the sulfoxy  
 15 group.  
 More advantageously still it is (S)-2',3',5'-trimethyl-  
 4'-hydroxy- $\alpha$ -dodecylthiophenyl- acetanilide (F12511).  
 Since the compounds of formula I can possess centers of  
 asymmetry, the anilide derivative according to the  
 20 present invention may be one of the various  
 stereoisomers or enantiomers or a mixture thereof.  
 These derivatives and the way in which they are  
 prepared are described in patent application  
 FR 2 741 619.

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- By "anilide derivative generated by supercritical  
 fluid" is meant in the sense of the present invention  
 any anilide derivative as defined above which has  
 undergone a step of generation by supercritical fluid,  
 30 in other words a step allowing its specific surface  
 area to be increased by virtue of the use of the  
 supercritical fluid. Such a step advantageously  
 consists in an RESS or SAS process.

By "porous support" is meant in the sense of the present invention any appropriate porous support which is soluble in an aqueous medium. The porous support is advantageously selected from the group consisting of  
5 cyclodextrins and a mixture thereof. Advantageously the support is  $\gamma$ -cyclodextrin.

By "supercritical fluid" is meant in the sense of the present invention any fluid which is used at a  
10 temperature and a pressure greater than their critical value. Advantageously the fluid is  $\text{CO}_2$ .

By "static mode" is meant in the sense of the present invention a reaction or a method in which all of the  
15 reactants are combined simultaneously and the reaction is left to proceed. For example, in step (b) of the present invention, a cocrystallized powder, water, and supercritical  $\text{CO}_2$  are placed in an autoclave and left to react for 16 hours. The mass of product does not  
20 change during the reaction. Conversely, in dynamic mode, the reactants are supplied in accordance with the progress of the reaction or of production. In a dynamic mode there is often circulation of a fluid or stirring. The mass of product  
25 changes during production. In the method of the present invention step (a) is typically a dynamic phase.

By "intimate mixture" is meant in the sense of the present invention a mixture of A and B in which A and B  
30 are uniformly distributed within the mixture obtained.

In one particular embodiment the method according to the present invention is such that the porous support is generated by supercritical fluid and such that step  
35 (a) comprises the following steps:  
(a1) dissolving the anilide derivative and the porous support in an organic solvent, said organic solvent being soluble in the supercritical fluid,

- (a2) continuously contacting the solution obtained in step (a1) with said supercritical fluid, so as to effect controlled desolvation of the anilide derivative and the support, and to ensure their coacervation,
- 5 (a3) washing the complex thus formed by extracting the residual solvent using the supercritical fluid, then separating the solvent in the liquid state and the supercritical fluid in the gaseous state.
- Advantageously step (a) consists in a coprecipitation
- 10 of the anilide derivative and of the porous support by the SAS process.

In another embodiment the method according to the present invention is such that the anilide derivative,

15 before being used in step (a), is generated by the process comprising the following steps:

- (i) dissolving the anilide derivative in an organic solvent, said organic solvent being soluble in the supercritical fluid,
- 20 (ii) continuously contacting the solution obtained in step (i) with said supercritical fluid, so as to effect desolvation of the anilide derivative, and to ensure its coacervation,
- (iii) washing the particles of anilide derivative thus
- 25 formed by extracting the residual solvent using said supercritical fluid, then separating the solvent in the liquid state and the supercritical fluid in the gaseous state,
- and such that the porous support used in step (a) is in solid form.
- 30 Advantageously the anilide derivative, before being used in step (a), is generated by precipitation in accordance with the SAS process.

- 35 In a third embodiment the method according to the present invention is such that the anilide derivative, before being used in step (a), is generated by the process comprising the following steps:

- (i) extracting the anilide derivative with the supercritical fluid, optionally admixed with a cosolvent,
- 5 (ii) vaporizing the supercritical mixture so as to effect desolvation of the anilide derivative, and to ensure its coacervation,
- (iii) washing the particles of anilide derivative thus formed with the supercritical fluid, then optionally separating the cosolvent in the liquid state and the
- 10 supercritical fluid in the gaseous state, and such that the porous support used in step (a) is in solid form.
- Advantageously the anilide derivative, before being used in step (a), is generated by precipitation in
- 15 accordance with the RESS process.

- In a fourth embodiment the method according to the present invention is such that step (a) comprises the following steps:
- 20 (a1) dissolving the anilide derivative in an organic solvent, said organic solvent being soluble in the supercritical fluid,
- (a2) continuously contacting the solution thus obtained with the supercritical fluid, so as to effect
- 25 desolvation of the anilide derivative, and to ensure its coacervation on the porous support placed in the reactor beforehand,
- (a3) washing the complex thus formed by extracting the residual solvent using the supercritical fluid, then
- 30 separating the solvent in the liquid state and the supercritical fluid in the gaseous state.
- Advantageously step (a) consists in the precipitation of the anilide derivative on the porous support by the SAS process.

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In a fifth embodiment the method according to the present invention is such that step (a) comprises the following steps:

(a1) extracting the anilide derivative with a supercritical fluid, optionally admixed with a cosolvent,

5 (a2) vaporizing the supercritical mixture so as to effect desolvation of the anilide derivative, and to ensure its coacervation on the porous support placed in the reactor beforehand,

(a3) washing the complex thus formed with the supercritical fluid, then optionally separating the  
10 cosolvent in the liquid state and the supercritical fluid in the gaseous state.

Advantageously step (a) consists in the precipitation of the anilide derivative on the porous support by the RESS process.

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Advantageously the organic solvent or the cosolvent is selected from the group consisting of alcohols, in particular methanol or butanol, ketones, in particular acetone, methyl ethyl ketone, cyclohexanone or N-methylpyrrolidone, acetic acid, ethyl acetate,  
20 dichloromethane, acetonitrile, dimethylformamide, dimethyl sulfoxide (DMSO), and a mixture thereof. Advantageously the solvent or cosolvent is ethanol or dimethyl sulfoxide.

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Advantageously step (b) of molecular diffusion of the method according to the present invention is performed with stirring.

More advantageously still step (b) of molecular  
30 diffusion of the method according to the present invention is performed in the presence of a diffusion agent.

A diffusion agent is for the purposes of the present invention any solvent which promotes interaction of the  
35 anilide derivative with the support.

Advantageously this diffusion agent is selected from the group consisting of alcohol, water with or without surfactant, and mixtures thereof. More advantageously still the agent is water.

This diffusion agent may be added continuously or discontinuously.

The time required for the molecular diffusion of step (b) is determined by any appropriate method. This step

- 5 (b) may be repeated as many times as desired in order to obtain a satisfactory dissolution rate. Advantageously step (b) lasts approximately 16 hours.

The pressure and temperature conditions of step (b) are selected so as to promote molecular diffusion.

- 10 Advantageously the pressure of the supercritical fluid is between 10 MPa and 40 MPa and the temperature is between 0 and 120°C.

More advantageously still the supercritical fluid is used at a pressure of between 10 MPa and 40 MPa and at

- 15 a temperature of between 0 and 120°C in all the steps of the method according to the present invention.

Each of the steps of the method according to the present invention is advantageously implemented in a closed reactor, in particular an autoclave.

- 20 Advantageously the method according to the present invention is performed continuously.

The present invention likewise provides a compound of interaction of an anilide derivative with a porous  
25 support, characterized in that it is obtainable by the method according to the present invention.

Advantageously the interaction compound according to the present invention is such that the anilide derivative thus complexed has a solubility in 5%  
30 aqueous sodium lauryl sulfate solution of greater than approximately 600 µg/ml.

The present invention also concerns an interaction compound according to the present invention as a drug, advantageously intended to treat dyslipidemia such as  
35 hypercholesterolemia and/or to prevent arteriosclerosis. It concerns also the use of an interaction compound according to the present invention to manufacture a drug intended to treat dyslipidemia



such as hypercholesterolemia and/or to prevent arteriosclerosis.

5 Physical characteristics of the powders in the various steps:

Active principle powder obtained by RESS:

- extremely light and pulverulent powder,
- size and type of monodisperse crystals: rodlets
- 10 with a length of 1-3  $\mu\text{m}$  and a diameter of 100 to 200 nm,
- bulk density of 12  $\text{kg/m}^3$ .

Active principle powder obtained by SAS:

- 15 - very light and pulverulent powder,
- size and type of monodisperse crystals: rodlets with a length of 10-20  $\mu\text{m}$  and a diameter of 100 nm,
- bulk density of 97  $\text{kg/m}^3$ .

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Cocrystallized powder (active principle/cyclodextrin)

- fine, light and pulverulent powder,
- bulk density 176  $\text{kg/m}^3$

25 Cocrystallized powder, aged (active principle/cyclodextrin)

- dense and nonpulverulent powder,
- bulk density 639  $\text{kg/m}^3$ .

30 Other subjects and advantages of the invention will become apparent for the skilled worker from the detailed description below and by means of references to the illustrative drawings which follow.

35 Figure 1 represents an SEM photo with an enlargement of 1000 $\times$  of the product F12511 obtained after crystallization and drying by conventional means.

Figure 2 represents an SEM photo with an enlargement of 2000× of the product F12511 obtained after crystallization and drying by conventional means.

5 Figure 3 represents an SEM photo with an enlargement of 1000× of the complex obtained after coprecipitation by the SAS process and washing with supercritical CO<sub>2</sub> of a solution of the product F12511 and  $\gamma$ -cyclodextrin in DMSO.

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Figure 4 represents an SEM photo with an enlargement of 2000× of the complex obtained after coprecipitation by the SAS process and washing with supercritical CO<sub>2</sub> of a solution of the product F12511 and  $\gamma$ -cyclodextrin in DMSO.

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Figure 5 shows an SEM photo with an enlargement of 1000× of the same complex as figures 3 and 4 after 16 hours of molecular diffusion in supercritical medium, in the presence of water.

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Figure 6 shows an SEM photo with an enlargement of 2000× of the same complex as figures 3 and 4 after 16 hours of molecular diffusion in supercritical medium, in the presence of water.

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Figure 7 shows a histogram of the bioavailability of the product F12511 according to the formulation used (compound of interaction with  $\gamma$ -cyclodextrin according to the method of the present invention or crystallized product F12511) in the dog.

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The method according to the invention includes in particular a step of molecular diffusion in supercritical medium, which allows a high level of interaction of the particles of anilide derivative in the envisioned support, as shown by the photos taken with the scanning electron microscope (figures 1 to 6). In these photos it can be seen that the structure of

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the compound is totally modified during the diffusion. Moreover, the dissolution in aqueous medium is also modified.

- 5 Accordingly the compound according to figures 1 and 2 has a solubility after 2 hours of 6 µg/ml in 5% aqueous sodium lauryl sulfate solution.
- The complex according to figures 3 and 4 has a solubility after 2 hours of 86 µg/ml in 5% aqueous sodium lauryl sulfate solution.
- 10 The complex according to figures 5 and 6 has a solubility after 2 hours of 516 µg/ml in 5% aqueous sodium lauryl sulfate solution.
- 15 The objective during this diffusion step is to improve the dissolution of the microparticles of active substance.

- The following step, which is a step of washing with supercritical fluid, further makes it possible to enhance the dissolution rate of the compound of interaction of the anilide derivative in the porous support.
- 20

- 25 Dissolution after two hours in aqueous medium is multiplied by approximately 100 by the method according to the present invention.

- The examples which follow of how the method is implemented are given by way of indication and not limitation.
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#### **Powder analysis protocols**

##### **Dissolution tests on product F12511**

- 35 **Operating conditions:**

Spectrophotometric detector set at 220 nm.

C8 graft column (Lichrospher 60RP-Select B), dimensions 25 × 0.4 cm, particle size: 5 µm.

Mobile phase:

* Acetonitile	820 ml
* Purified water	180 ml
* Glacial acetic acid	1 ml

Flow rate: 1 ml/min

5 Preparation of solutions:

**Solution under examination**

Introduce an amount of complex corresponding to approximately 100 mg of product F12511 into 100 ml of 5% (m/V) sodium lauryl sulfate in H<sub>2</sub>O. Subject the  
10 system to magnetic stirring in a waterbath at 37°C ± 0.5°C. Withdraw 2 ml sample of this suspension after 2 hours of stirring and filter it on a Gelman GHP Acrodisc GF (R) filter.

Dilute the samples 1/5 in the mobile phase.

15 Carry out 2 tests.

**Control solution**

Introduce 8 mg of reference product F12511 (starting material used to prepare the complex) in a 100 ml flask  
20 and dissolve it in 1 ml of tetrahydrofuran (THF). Make up to volume with the mobile phase.

**Range**

	T1	T2	T3	T4	T5
Control solution (ml)	0.5	1.5	2.0	3.0	4.0
Mobile phase	qs 20 ml				
Concentration (µg/ml)	2.0	6.0	8.0	12.0	16.0

**Test procedure:**

25 Inject 20 µl of each control solution. Measure the area of the peak of product F12511 and represent its variation as a function of concentration in the form of a graph. The correlation coefficient is > 0.995. Inject 20 µl of the test solution. Measure the area of the  
30 peak of product F12511 present in the test solution, and ensure that it lies between that of T1 and of T5 in the range.

If this is not the case, perform a dilution in the solubilizing solvent and/or adjust the injection volume  
35 of the test solution.

From this, work out the concentration X ( $\mu\text{g/ml}$ ) of the test solution.

Calculate the amount of dissolved product F12511 in mg/ml by the following formula:

$$5 \quad \frac{X \times 20 \times F \times 5}{1000 \times Y}$$

Y: injection volume of the test solution

F: dilution factor

#### Measurements of specific surface areas

- 10 The specific surface area measurements were carried out on a BET ASAP 2010 adsorption apparatus from Micrometrics.

#### Sample preparation

- Before the measuring phase, the sample requires a degassing step. This step consists in evacuating the cell containing the sample until a vacuum of at least 0.003 mm Hg, or approximately 0.004 mbar, is reached stably. This degassing is carried out at a temperature of 50°C (duration: approximately 16 hours).
- 20 At the end of degassing, the cell containing the sample is filled with helium and transferred to the measuring station, where evacuation is repeated before analysis.

#### Processing of the adsorption isotherms

- 25 The specific surface area was determined in accordance with the BET theory, i.e., in accordance with the following relationship:

$$\frac{1}{W \cdot [(P_0/P) - 1]} = \frac{1}{C W_m} + \frac{C - 1}{W_m \cdot C} \cdot (P/P_0)$$

30

W: volume of gas adsorbed (under standard temperature and pressure (STP) conditions) per unit mass of sample.

W<sub>m</sub>: volume of gas adsorbed (under STP conditions) in a monolayer per unit mass of sample.

35 P<sub>0</sub>: saturation pressure.

C: constant.

The isotherm is then plotted as follows:

$$\frac{1}{W \cdot [(P_0/P) - 1]}$$

- 5 As a function of  $P/P_0$ : we then have a straight line of which the slope and the ordinate at the origin give us C and  $W_m$ .

The specific surface area is then given by the following formula:

10  $a \text{ (m}^2 \cdot \text{g}^{-1}) = N_m N_A E$

E: space occupancy of the nitrogen molecule. For nitrogen at 77 K operating temperature this is generally taken to be  $E = 0.162 \text{ nm}^2$ .

$N_A$ : Avogadro's number.

- 15  $N_m$ : number of moles of nitrogen adsorbed on a monolayer per unit mass of sample, calculated from  $W_m$ .

The measurements are carried out within a conventional field of relative pressure in which the BET theory is valid, namely  $0.05 < P/P_0 < 0.2$ . In order to verify the

- 20 validity of this theory, one practical means is to look at the direction in which the quantity  $N_{\text{adsorbed}} \cdot (1 - P/P_0)$  changes as a function of  $P/P_0$ : it should increase continually with  $P/P_0$ .

- 25 Verify the range of applicability of the BET theory in this way, and if necessary readjust the range of relative pressures.

#### Comparative example 1: precipitation by SAS/DMSO of product F12511

30

A 150 ml solution of the product F12511 in DMSO with a concentration of 115 g/l is precipitated continuously by the solvent-antisolvent (SAS) process, in the presence of  $\text{CO}_2$ , in a 2 l autoclave equipped with a

35 1.37 l basket. The flow rate of the solvent pump is 0.6 ml/min. The temperature and pressure within the autoclave are selected so as to give a  $\text{CO}_2$  density of

- 0.8. After approximately 130 ml of solution have been precipitated the injection of the solute and then the injection of CO<sub>2</sub> are stopped, and washing is carried out by passage of a flow of CO<sub>2</sub> (300 bar, 50°C) for 3 hours. The autoclave is subsequently depressurized. The yield of this step is 87%.

Nature of powder	Dissolution (µg/ml)	BET (m <sup>2</sup> /g)
F12511	6-12	14
F12511 precipitated by SAS	62	54

10 **Comparative example 2: precipitation by RESS of product F12511**

- 10 g of product F12511 are placed in an autoclave and extracted with supercritical CO<sub>2</sub> at 100°C and 265 bar. The fluid is then precipitated in a second chamber, and 0.6 g of product F12511 is recovered. Measurements are made of the dissolution after two hours and of the specific surface area:

Nature of powder	Dissolution (µg/ml)	BET (m <sup>2</sup> /g)
F12511	12	14
F12511 precipitated by RESS	76	67

20 **Comparative example 3: coprecipitation of product F12511 and γ-cyclodextrin by SAS/DMSO**

- A 150 ml solution of product F12511 (concentration: 57.5 g/l) and γ-cyclodextrin (concentration of 172.5 g/l) in DMSO is precipitated continuously by the solvent-antisolvent (SAS) process, in the presence of CO<sub>2</sub>, in a 2 l autoclave equipped with a 1.37 l basket. The flow rate of the solvent pump is 0.4 ml/min. The temperature and pressure within the autoclave are selected so as to give a CO<sub>2</sub> density of 0.9. After approximately 100 ml of solution have been precipitated, the injection of the solute and then the

injection of CO<sub>2</sub> are stopped, and the powder obtained is washed by passage of a flow of CO<sub>2</sub> (300 bar, 50°C) for 2 hours. The autoclave is subsequently depressurized.

- 5 The yield of this step is 81%.

The results of the dissolution measurements are collated in the table below:

Nature of powder	Dissolution (µg/ml)
F12511	12
F12511 coprecipitated by SAS/DMSO	100

- 10 Example 4: Coprecipitation, inclusion, and washing starting from a solution of product F12511 and γ-cyclodextrin in DMSO

- A 450 ml solution of product F12511 (concentration: 15 40 g/l) and γ-cyclodextrin (concentration of 240 g/l) in DMSO is precipitated continuously with a solvent-antisolvent (SAS) process, in the presence of CO<sub>2</sub>, in a 6 l autoclave equipped with 4 l basket. The flow rate of the solvent pump is 1.1 ml/min. The temperature and 20 pressure within the autoclave are selected so as to give a CO<sub>2</sub> density of 0.9 ± 0.05. After approximately 450 ml of solution have been precipitated, the injection of the solute and the injection of CO<sub>2</sub> are stopped, and the system is let down gently, so as not 25 to liquefy the supercritical fluid.

The average yield of this step is 94%.

- The powder coprecipitated in the preceding step is mixed with osmosed water (mass ratio of 25% of water), and the mixture is placed in the 4 L Poral basket, 30 which in turn is placed in the 6 l precipitation autoclave.

- The autoclave is closed and the system is pressurized with supercritical CO<sub>2</sub> so as to give a static pressure of 300 bar, and a temperature of 65°C within the 35 autoclave.



After one night of molecular diffusion the autoclave is let down gently, and this step is repeated, without adding diffusion agent (water), for one night.

- 5 The complex thus obtained is then washed with a flow of supercritical CO<sub>2</sub> (270 bar, 40°C) for 8 hours. Letdown is followed by a dissolution measurement on the resulting powder.

Nature of powder	Dissolution (µg/ml)
F12511 before coprecipitation	~ 15
F12511/γ-cyclodextrin compound after molecular diffusion	440
F12511/γ-cyclodextrin compound after molecular diffusion, and washed	662

- 10 These results show the advantage of a method combining coprecipitation, inclusion, and washing in supercritical medium for improving the dissolution of the anilide derivative in aqueous medium.
- 15 Pharmacokinetic tests on dogs were carried out with an F12511/γ-cyclodextrin interaction compound obtained by this method. Standardized doses of 3 mg/kg were administered to 5 dogs, and the plasma concentration (expressed in ng/ml.h) of F12511 was measured. The
- 20 results relating to the F12511 obtained after crystallization and drying by a conventional route and those relating to the F12511/γ-cyclodextrin interaction compound obtained by the above-described method of the present invention are shown in the histogram of
- 25 figure 7.

- It is found that the administration of doses prepared from the F12511/γ-cyclodextrin interaction compound obtained by the method according to the present
- 30 invention makes it possible to improve bioavailability in the dog by a factor of 10.

**Comparative example 5: Precipitation and inclusion in  $\gamma$ -cyclodextrin of product F12511 generated by SAS process/ethanol**

- 5 An 8 l solution of product F12511 (concentration: 5 g/l) in ethanol is precipitated continuously with the solvent-antisolvent (SAS) process, in the presence of CO<sub>2</sub>, in a 6 l autoclave equipped with a 4 l basket. The flow rate of the solvent pump is 41.7 ml/min. The
- 10 temperature and pressure within the autoclave are selected so as to give a CO<sub>2</sub> density of 0.8. After approximately 8 l of solution have been precipitated, the injection of the solute and the injection of CO<sub>2</sub> are stopped, and the system is let down gently, so as
- 15 not to liquefy the supercritical fluid.
- The 4.3 g of the anilide derivative precipitated in the preceding step are mixed with 25.8 g of  $\gamma$ -cyclodextrin and 10 g of osmosed water, and the mixture is placed in the 4 l Poral basket, which in turn is placed in the
- 20 6 l precipitation autoclave.
- The autoclave is closed and the system is pressurized with supercritical CO<sub>2</sub> so as to give a static pressure of 300 bar, and a temperature of 65°C within the autoclave.
- 25 Letdown is carried out after 16 hours of molecular diffusion.

Nature of powder	Dissolution ( $\mu$ g/ml)
F12511 before precipitation	~ 15
F12511 precipitated with supercritical CO <sub>2</sub>	80
F12511/ $\gamma$ -cyclodextrin compound after molecular diffusion	155

- Comparative example 6: Precipitation and inclusion in  $\gamma$ -cyclodextrin of product F12511 generated by SAS process/DMSO**
- 30

- A 150 ml solution of product F12511 (concentration: 200 g/l) in DMSO is precipitated continuously with the solvent-antisolvent (SAS) process, in the presence of CO<sub>2</sub>, in a 2 l autoclave equipped with a 1.37 l basket.
- 5 The flow rate of the solvent pump is 0.5 ml/min. The temperature and pressure within the autoclave are selected so as to give a CO<sub>2</sub> density of 0.9. After approximately 135 ml of solution have been precipitated, the injection of the solute and the
- 10 injection of CO<sub>2</sub> are stopped, and the system is let down gently, so as not to liquefy the supercritical fluid.
- The 1 g of the anilide derivative precipitated in the preceding step is mixed with 6 g of  $\gamma$ -cyclodextrin and
- 15 2.33 g of osmosed water, and the mixture is placed in the 1.37 l Poral basket, which in turn is placed in the 2 l precipitation autoclave.
- The autoclave is closed and the system is pressurized with supercritical CO<sub>2</sub> so as to give a static pressure
- 20 of 300 bar, and a temperature of 100°C within the autoclave.
- Letdown is carried out after 16 hours of molecular diffusion.

Nature of powder	Dissolution ( $\mu$ g/ml)
F12511 before precipitation	5
F12511 precipitated with supercritical CO <sub>2</sub>	57
F12511/ $\gamma$ -cyclodextrin compound after molecular diffusion	165

25

**Comparative example 7: inclusion in  $\gamma$ -cyclodextrin of product F12511 generated by RESS process**

- 40 g of product F12511 are placed in a 4 l basket which
- 30 in turn is placed in a 6 l autoclave. The active substance is extracted with a supercritical mixture of CO<sub>2</sub> and ethanol (5% by mass) and the substance is

precipitated at 120 bar and 55°C. After 3 hours, the injections of CO<sub>2</sub> and of ethanol are stopped.

8.96 g of the anilide derivative precipitated in the preceding step are mixed with 53.76 g of  $\gamma$ -cyclodextrin and 20.87 g of osmosed water, and the mixture is placed in the 4 l Poral basket, which in turn is placed in the 6 l precipitation autoclave.

The autoclave is closed and the system is pressurized with supercritical CO<sub>2</sub> so as to give a static pressure of 300 bar, and a temperature of 65°C within the autoclave.

The autoclave is letdown gently after 16 hours of molecular diffusion.

Nature of powder	Dissolution ( $\mu$ g/ml)
F12511 before precipitation	~ 10
F12511 precipitated with supercritical CO <sub>2</sub>	8
F12511/ $\gamma$ -cyclodextrin compound after molecular diffusion	292

#### Summary of results

The table below summarizes the different methods employed and also the corresponding dissolution results, and permits the deduction therefrom of the method most suitable for the manufacture of F12511 product with high dissolution in aqueous medium:

Method	Comp. Ex. 1	Comp. Ex. 2	Comp. Ex. 3	Ex. 4	Ex. 4	Comp. Ex. 5	Comp. Ex. 5
Precipitation * by RESS		X					
Precipitation * by SAS/DMSO	X						
Coprecipitation ** by SAS/DMSO			X	X	X		
Precipitation * by SAS/EtOH						X	X
Conventional crystallization							
Stirred molecular diffusion							
Non-stirred molecular diffusion				X	X		X
Washing	X		X		X		
Dissolution (µg/ml)	62	76	100	440	662	80	155

Method	Comp. Ex. 6	Comp. Ex. 6	Comp. Ex. 7	Comp. Ex. 7	Ex. 8	Ex. 8
Precipitation * by RESS			X	X		
Precipitation * by SAS/DMSO	X	X				
Coprecipitation ** by SAS/DMSO						
Precipitation * by SAS/EtOH						
Conventional crystallization					X	X
Stirred molecular diffusion						X
Non-stirred molecular diffusion		X		X	X	
Washing	X					
Dissolution (µg/ml)	57	165	8	292	124	334

\* Precipitation of product F12511 alone

\*\* Coprecipitation of a solution of product F12511 and

- In light of these results it is clear that the method which allows the greatest dissolution of product F12511 in an aqueous medium to be obtained is the method
- 5 combining the steps of generating product F12511 using supercritical fluid, advantageously by coprecipitation of product F12511 and  $\gamma$ -cyclodextrin, molecular diffusion in static mode, advantageously with stirring, and washing.

10

Comparative tests 9:

- To validate the fact that it is indeed the method as a whole that allows us to obtain the end results, and not
- 15 one of the intermediate steps, we carried out dissolution tests as described above on various mixtures and obtained the following results:

	Before diffusion	After diffusion
F12511/ $\gamma$ -cyclodextrin Crude powders Physical mixture	19 $\mu\text{g/ml}$	142 $\mu\text{g/ml}$
F12511/ $\gamma$ -cyclodextrin Powders crystallized by SAS Separately Physical mixture	69 $\mu\text{g/ml}$	150 $\mu\text{g/ml}$
F12511/ $\gamma$ -cyclodextrin Cocrystallized powders	100 $\mu\text{g/ml}$	671 $\mu\text{g/ml}$

20

**CLAIMS**

1. A method for preparing compounds of interaction of an anilide derivative with a porous support, characterized in that it comprises the following steps:
- 5 (a) mixing the anilide derivative generated by supercritical fluid and the defined amount of porous support,
- (b) implementing a step of molecular diffusion by
- 10 contacting in static mode a supercritical fluid with the mixture obtained in step (a) for the time required to improve the dissolution in an aqueous medium of the mixture obtained in step (a),
- (c) washing the interaction compound obtained in step
- 15 (b) with a flow of supercritical fluid,
- (d) recovering the particles of the interaction compound thus formed.
2. The method according to claim 1, characterized in
- 20 that the porous support is generated by supercritical fluid and in that step (a) comprises the following steps:
- (a1) dissolving the anilide derivative and the porous support in an organic solvent, said organic solvent
- 25 being soluble in the supercritical fluid,
- (a2) continuously contacting the solution obtained in step (a1) with said supercritical fluid, so as to effect controlled desolvation of the anilide derivative and the support, and to ensure their coacervation,
- 30 (a3) washing the complex thus formed by extracting the residual solvent using the supercritical fluid, then separating the solvent in the liquid state and the supercritical fluid in the gaseous state.
- 35 3. The method according to claim 1, characterized in that the anilide derivative, before being used in step (a), is generated by the process comprising the following steps:

- (i) dissolving the anilide derivative in an organic solvent, said organic solvent being soluble in the supercritical fluid,
- (ii) continuously contacting the solution obtained in  
5 step (i) with said supercritical fluid, so as to effect desolvation of anilide derivative, and to ensure its coacervation,
- (iii) washing the particles of anilide derivative thus formed by extracting the residual solvent using said  
10 supercritical fluid, then separating the solvent in the liquid state and the supercritical fluid in the gaseous state, and in that the porous support used in step (a) is in solid form.
4. The method according to claim 1, characterized in that the anilide derivative, before being used in step (a), is generated by the process comprising the following steps:
- (i) extracting the anilide derivative with the  
20 supercritical fluid, optionally admixed with a cosolvent,
- (ii) vaporizing the supercritical mixture so as to effect desolvation of the anilide derivative, and to ensure its coacervation,
- (iii) washing the particles of anilide derivative thus  
25 formed with the supercritical fluid, then optionally separating the cosolvent in the liquid state and the supercritical fluid in the gaseous state, and in that the porous support used in step (a) is in solid form.
5. The method according to claim 1, characterized in that the step (a) comprises the following steps:
- (a1) dissolving the anilide derivative in an organic solvent, said organic solvent being soluble in the  
35 supercritical fluid,
- (a2) continuously contacting the solution thus obtained with the supercritical fluid, so as to effect desolvation of the anilide derivative, and to ensure



its coacervation on the porous support placed in the reactor beforehand,

- (a3) washing the complex thus formed by extracting the residual solvent using the supercritical fluid, then  
5 separating the solvent in the liquid state and the supercritical fluid in the gaseous state.

6. The method according to claim 1, characterized in that step (a) comprises the following steps:

- 10 (a1) extracting the anilide derivative with a supercritical fluid, optionally admixed with a cosolvent,  
(a2) vaporizing the supercritical mixture so as to effect desolvation of the anilide derivative, and to  
15 ensure its coacervation on the porous support placed in the reactor beforehand,  
(a3) washing the complex thus formed with the supercritical fluid, then optionally separating the cosolvent in the liquid state and the supercritical  
20 fluid in the gaseous state.

7. The method according to any one of claims 2 to 6, characterized in that the organic solvent or cosolvent is selected from the group consisting of alcohols,  
25 ketones, acetic acid, ethyl acetate, dichloromethane, acetonitrile, dimethylformamide, dimethyl sulfoxide, and a mixture thereof.

8. The method according to any one of the preceding  
30 claims, characterized in that the supercritical fluid is CO<sub>2</sub>.

9. The method according to any one of the preceding claims, characterized in that the anilide derivative is  
35 the (S)-2',3',5'-trimethyl-4'-hydroxy- $\alpha$ -dodecylthiophenylacetanilide.

10. The method according to any one of the preceding claims, characterized in that the porous support is

selected from the group consisting of cyclodextrins and a mixture thereof.

11. The method according to any one of the preceding  
5 claims, characterized in that step (b) of molecular diffusion is carried out with stirring.

12. The method according to any one of the preceding  
claims, characterized in that step (b) of molecular  
10 diffusion is carried out in the presence of a diffusion agent.

13. The method according to claim 12, characterized in  
that the diffusion agent is selected from the group  
15 consisting of alcohol, water with or without surfactant, and mixtures thereof.

14. The method according to any one of the preceding  
claims, characterized in that the pressure of the  
20 supercritical fluid is between 10 MPa and 40 MPa and the temperature is between 0 and 120°C.

15. The method according to any one of the preceding  
claims, characterized in that each of the steps of the  
25 method is implemented in a closed reactor, in particular an autoclave.

16. The method according to any one of the preceding  
claims, characterized in that it is carried out  
30 continuously.

17. A compound of interaction of an anilide derivative  
in a porous support, characterized in that it is  
obtainable by the method according to any one of  
35 claims 1 to 16.

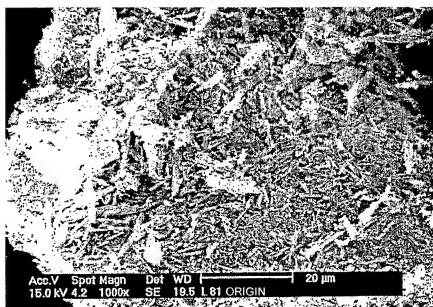
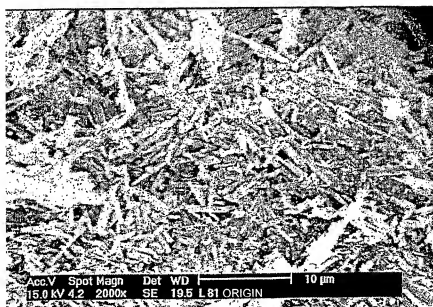
18. The compound according to claim 17, characterized  
in that the anilide derivative thus complexed has a

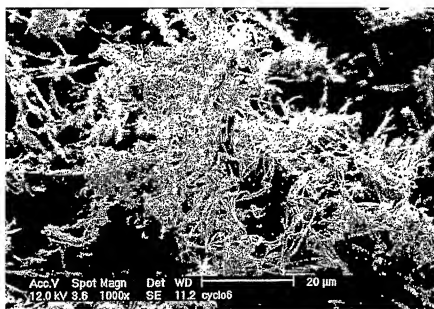
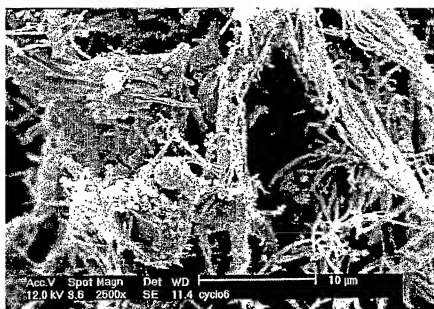
solubility in 5% aqueous sodium lauryl sulfate solution  
of greater than approximately 600 µg/ml.

19. The compound according to any of claims 17 or 18  
5 as a drug.

20. The compound according to claim 19 as a drug  
intended to treat dyslipidemia such as  
hypercholesterolemia and/or to prevent  
10 arteriosclerosis.

21. Use of the compound according to any of claims 17  
or 18 for the manufacture of a drug intended to treat  
dyslipidemia such as hypercholesterolemia and/or to  
15 prevent arteriosclerosis.

FIG.1FIG.2

FIG.3FIG.4